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HUMAN CYSTIC DUCT: STRUCTURE AND FUNCTION IN GALLSTONE DISEASE

by

FINBAR LENNON

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled HUMAN CYSTIC DUCT: STRUCTURE AND FUNCTION IN GALLSTONE DISEASE submitted by FINBAR LENNON in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN EXPERIMENTAL SURGERY.

DEDICATION

In Memory of Dad

A B S T R A C T

This research assessed the effects of gallstone disease upon the structure and motility of the human cystic duct.

Freshly removed human gallbladders and cystic ducts were examined by a pathologist and portions were removed immediately for (i) histological examination of the gallbladder body and neck and the cystic duct (ii) measurement of the muscle layer in all three areas, and (iii) pharmacological studies of motility in organ baths. Canine tissue from the gallbladder body and cystic duct was likewise examined.

The histology specimens were coded 'blind' (observer unaware of the results of the motility studies) and were classified according to the degree of inflammation. The morphology of the muscle layer was studied in transverse sections, with particular attention to the thickness of the layer.

Contractile responses were characterized, using standard organ bath techniques, to cholinergic stimulation; cholecystokinin; and the mediators of inflammation histamine, and bradykinin.

Histological examination of the human gallbladders differentiated three stages of disease; mild chronic, advanced chronic, and acute cholecystitis. The histological abnormalities were qualitatively the same in the gallbladder body and neck in the three disease stages. Histological scores and pathological classification of the cystic ducts were identical with those of the corresponding gallbladders.

In both human and canine tissues the muscle layer was thickest in the gallbladder body, thinner in the gallbladder neck (human), and thinnest in the cystic duct.

The cystic duct responses in the human and the dog were always contractile. The sensitivity to acetylcholine and histamine did not differ between the gallbladder and cystic duct in either species. The sensitivity to cholecystokinin was 3 times less in the human cystic ducts than in the gallbladders. The sensitivity to bradykinin was greater in the human gallbladder neck and cystic duct than in the gallbladder body, whereas in the dog there was no such difference. In general, the magnitude of the contractile responses to all agonists was greatest in the gallbladder body > gallbladder neck > cystic duct.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Traditionally, and even in the current surgical literature (1,2) it is assumed that the major regulators of extrahepatic bile flow are the gallbladder and the sphincter of Oddi. Hardly any attention has been given to the possibility that the cystic duct could or does regulate bile flow. It has generally been regarded only as a passive conduit. Now, however, largely as a result of reports by Scott and co-workers (3-5) and Pitt et al. (6,7), the role of the cystic duct in gallbladder filling and emptying and its possible contribution to gallstone formation are receiving more attention.

Gallbladder filling and emptying result from the dynamic interaction of the gallbladder, cystic duct, and sphincter of Oddi. This intricate mechanism appears to differ in health and disease. In health the tone of the gallbladder and resistance of the sphincter of Oddi probably are the main determinants of gallbladder filling and emptying (8,9). Although the cystic duct has sphincter-like properties (10,11) and responds to pharmacological and hormonal stimuli, resistance to flow is normally lower through this than through the sphincter of Oddi (4). Thus, the duct may not be active in extrahepatic biliary dynamics in health. In pre-gallstone disease in prairie dogs (Cynomys ludivicianus), however, when lithogenic bile is present, the cystic duct affects biliary mechanics. At that stage the gallbladder's tone and its response to cholecystokinin (CCK) remain unchanged (12-14); in fact, even with prolonged cholesterol feeding, when the gallbladder is filled

with stones and mucus, neither resistance in the sphincter of Oddi nor the response to CCK appears affected (6,12). The only change in pre-gallstone disease is increasing resistance to flow through the cystic duct (7) with increasing lithogenicity of the bile (7). This must be a major cause, if not the prime cause, of stasis in gallbladder contents. This scheme, which accords the cystic duct a major role in gallstone formation, has renewed interest in the theory that gallbladder stasis is a prime etiological factor in gallstone formation (15). Once stasis has begun, changes undoubtedly occur in gallbladder tone, leading to further alterations in gallbladder--cystic-duct dynamics. The increased resistance noted in the cystic duct in the pre-gallstone state in prairie dogs may also occur in man.

The cause of this change in cystic-duct resistance in pre-gallstone disease is unknown. Possible organic causes include the secretion of excess mucus by the mucosa of both gallbladder and cystic duct (16), which could increase bile viscosity in the cystic duct and thus increase resistance, and mechanical obstruction of the duct. Partial or complete obstruction has been observed frequently in patients with cholelithiasis (17), and anomalies and narrowing of the duct have been reported in up to 65% of humans (18,19). Cole and associates suggested that partial obstruction of the duct may be the cause of cholecystitis and, by obstruction of the cystic duct in animals, produced gallbladder lesions typical of chronic cholecystitis in human gallbladders (17). However, although narrowing of the cystic duct always delays gallbladder emptying, it does not invariably lead to gallstone formation (20). Alternatively, change in resistance of the cystic duct could have a functional cause, for example, a differential alteration in the

sensitivity of the duct and gallbladder to neuro-hormonal stimulation by agents (e.g., CCK and vasoactive amines) that help regulate extrahepatic biliary motility.

ANATOMY

General

The cystic duct joins the gallbladder to the extrahepatic duct system. The junction is usually at the right lateral aspect of the common hepatic duct, but can occur at any point between the porta hepatis and the ampullary region. Its mode of termination may be angular, parallel, or spiral. An angular insertion occurs in approximately 65% of cases; in 25% of cases the cystic duct and common hepatic duct run a parallel course for some distance before joining; and in 10% of cases a spiral insertion occurs (21). The cystic duct may be short and straight or long and tortuous, averaging 2.0 to 3.0 cm long (22,23) in the range of 0.4 to 6.5 cm (18,22-24). Its diameter varies from 0.1 to 0.9 cm (22). It contains prominent mucosal folds, called spiral folds or valves of Heister. Numerous attempts have been made to determine the function of the folds; some workers have suggested that they act as unidirectional valves in regulating bile flow (25) but most agree with Scott and Otto (3) that they do not. The wall of the cystic duct consists of three layers similar to those of the gallbladder--mucosal, fibromuscular, and areolar--and in the submucosal region contains a thin layer of muscle that appears continuous with the muscle layer of the gallbladder. There appears to be no anatomic sphincter at the junction of the gallbladder and cystic duct (26,27), as was proposed by Lütkens (28), but the amount of muscle appears to be greater at the cholecystic junction and proximally in the duct than in the mid and distal parts of the duct (29). This variation may cause a differential

motor response along the duct and thus may have a physiological sphincter effect.

Neuroanatomy

Alexander (30) reviewed the literature on the extrinsic innervation of the extrahepatic biliary tract. The gallbladder and the biliary ducts are supplied by both the sympathetic and the parasympathetic nervous systems. He confirmed the presence of parasympathetic nerve cells and a three-layered interconnecting intramural plexus in the gallbladder, but failed to find a submucous supply in the common bile duct. Burnett et al. (31), who demonstrated the distribution and mode of termination of intramural ganglia, found a rich three-layered network comprising small, medium-sized, and large medullated nerves; nerve cells in large groups were seen in the gallbladder and cystic duct, but were less numerous in the common bile duct until the lower end was reached. These investigators concluded that the intrinsic nerve supply of the extra-hepatic biliary tract plays a vital part in its function.

PHYSIOLOGY

Pressures in the Extrahepatic Biliary Tract

The entry of food into the duodenum results in gallbladder contraction, forcing bile into the common bile duct. After relaxation of the sphincter of Oddi, bile enters the duodenum. The pressures within the extrahepatic biliary tract depend upon the rate and pressure of bile flow into the system and upon the resistance to flow through the system (8,32,33).

It has been shown experimentally that the liver can continuously secrete bile against an opposing pressure of between +10 and 30 cm H_2O, depending upon the period of fasting (34). This hepatic secretory pressure is significantly greater than the resting pressure (+10 cm) within the gallbladder, which appears to fill passively so long as the sphincter of Oddi maintains a fairly high resistance to flow. In the distinct high pressure zone at the choledochoduodenal junction, pressure is higher (maximum, 30 cm H_2O) and motor activity is different from that in the common duct or duodenum (35). The sphincter of Oddi appears to be the major control mechanism regulating gallbladder filling and emptying (8). When the gallbladder contracts, the pressure within it rises (to between +20 and 30 cm H_2O) and the sphincter relaxes (36). During fasting, 70% of the hepatic bile enters the gallbladder (37), an action that is maintained in patients whose gallstones are not obstructing the cystic duct.

Components of Extrahepatic Biliary-tract Motility

Gallbladder

One of the physiological mechanisms identified in the gallbladder is smooth-muscle contraction to discharge the bile into the upper part of the small intestine and relaxation during storage. The gallbladder's inherent tone and contractile activity constitute a complex mechanism that is influenced by autonomic innervation, hormonal responses, and locally released substances such as histamine and prostaglandins (38).

Cystic Duct

The muscle layer in the cystic duct is continuous with that of the gallbladder. It forms a loose meshwork and thus can exert tension in multiple axes, and most investigators support the view that it has sphincter-like properties (3,11,25,39). Potter and Mann (39) found that pressures in the gallbladder and common bile duct vary independently, and Doyle and Farrar (11), who demonstrated a pressure difference between these two organs, speculated that this is an effect of the cystic duct. In 1970 Torsoli et al. (25) observed in humans that the flow of contrast medium through the cystic duct stopped when morphine or CCK was given and that this was reversible with amyl nitrate, and in 1979 Scott and Otto (3) noted in dogs an increased resistance to flow through the cystic duct after morphine or CCK administration. Contractile and relaxant responses that can affect resistance to flow through the canine cystic duct have been produced with various neurotransmitter and hormonal agents (40).

All of these findings imply that the smooth muscle in the duct is responsible for the sphincter-like activity. The only dissenting opinion in the recent literature is that of Martin et al. (41), who suggested that the cystic duct probably acts as a "nondirectional pressure-relief valve" and that the mechanism may be nonsphincteric.

Common Bile Duct

It is still not clear whether the common bile duct (CBD) has an active or passive role in the propulsion of bile through its lumen. The CBD exhibits spontaneous rhythmical activity in vitro (42) and some investigators have reported peristalsis (43-45), but the latter may not

be significant (46,47). It seems that any activity in the CBD is due to the backward reflection of pressure fluctuations in the duodenum or sphincter of Oddi (48). Toouli and Watts (42) do not discount the possibility that the CBD has a regulatory role in bile flow, basing their opinion on the demonstration of contractile responses in the human and canine CBD to various stimuli *in vitro*. However, most investigators regard the CBD as only a passive conduit.

Sphincter of Oddi

This sphincter is the principal regulator of bile flow into the duodenum (49) and appears to be the major control mechanism regulating gallbladder filling and emptying, but its activity can be modified by the duodenal musculature (50). The sphincter's smooth muscle responds to various neural and hormonal stimuli. In humans, the hormone that most strongly affects it directly seems to be CCK; unlike its action in the gallbladder, which it contracts, CCK relaxes the sphincter of Oddi. It is unclear whether this effect in the sphincter is a result of smooth-muscle relaxation or whether, by enchancing smooth-muscle contraction and activating a pumping action, it facilitates the flow of bile into the duodenum. Whereas CCK increases the sphincter's activity in some other mammalian species (51,52), it depresses its contractility in humans (15,53): Toouli *et al*. (54) recently demonstrated that CCK octapeptide (CCK-OP) abolished the phasic contractions and reduced basal pressure. These investigators had postulated, based on studies in cats (54), that CCK-OP induces this decrease in resistance by stimulating

nonadrenergic, noncholinergic inhibitory nerves within the sphincter, and had inferred that this is CCK's dominant effect on the sphincter and overrides its direct stimulation of the muscle.

CONTROL OF CYSTIC-DUCT MOTOR FUNCTION

The control of extrahepatic biliary tract motility appears to be similar to that of other parts of the gastro-intestinal tract. It has a well-developed autonomic nerve supply and there is an intramural nervous plexus mainly in the gallbladder and cystic duct. There are also many hormones and locally released substances (e.g. histamine and prostaglandins) that are known to affect its motility. There have been very few studies on the cystic duct but the control of its motility appears to be the same as other parts of the biliary tract.

Neural Control

The cystic duct has a rich nerve supply, both extrinsic and intrinsic (31). Baumgarten and Lange (55) found higher concentrations of noradrenaline in the extrahepatic biliary tract of the rhesus monkey than any of the other catecholamines. They also showed that there were regional differences in its localization. The concentration of noradrenaline in the cystic duct was approximately twice as high as in the gallbladder body. Clanachan et al (40) have shown both adrenergic and cholinergic receptors in the cystic duct and others have demonstrated adrenergic receptors (56). Stimulation of α-adrenoceptors or muscarine receptors constrict the cystic duct, whereas stimulation of

β-adrenoceptors relaxes it (40).

Hormonal Control

Cholecystokinin

CCK contracts the gallbladder and relaxes the sphincter of Oddi in many species, including man. This action is physiological, in that gallbladder contraction occurs in response to endogenously released CCK (57). Peptides related to CCK (e.g., caerulein) have the same action; in fact, caerulein is 16 times more potent than CCK in experiments in dogs (58). The findings that CCK induced stimulation of gallbladder muscle contraction *in vitro* is not blocked by anticholinergic, anti-adrenergic, or depolarizing agents, or by tetrodotoxin (59-61), supports the belief that CCK acts directly on the smooth muscle, and thus implies an effect on cystic-duct muscle. Flow through the cystic duct decreased when CCK was given (3); however, the concentrations of CCK were probably beyond that encountered under physiological conditions. Recently it was shown that the reduction in flow through the intact duct and in the contraction of strips of duct in response to CCK is dose-related (62). This study also showed differential sensitivity to CCK in the canine gallbladder and cystic duct, and a slower response and slower rate of contraction of the cystic duct in response to CCK than of the gallbladder. These latter findings help to explain how CCK-induced constriction of the cystic duct does not prevent the outflow of bile from the gallbladder. If this differential sensitivity to CCK is present in man, it may be altered in disease; in gallstone disease, such an alteration might result in stasis in the gallbladder.

It is not known whether CCK stimulates inhibitory nerves in the cystic duct to override its direct stimulation of the duct's smooth muscle, an action that has been postulated for the sphincter of Oddi (54).

Mediators of Inflammation

Many substances within the gut wall have potent effects when applied to isolated segments of the muscle; thus, they may influence gut motility. Injury results in a corresponding degree of anoxia and acidification of the damaged tissue, together with increased proteolytic activity and inhibition of kininases; these events predispose to the release or activation of vaso-active agents such as histamine and kinins.

The present study sought to delineate the actions of histamine and bradykinin, substances that are associated with the inflammatory reaction and may play a part in cholecystitis. These agents can increase vascular permeability and induce smooth-muscle contraction.

Histamine

This biologically potent decarboxylated amino acid is of low molecular weight and has an imidazole ring. It is stored in basophils and mast cells; it is also present, unassociated with mast cells and subject to rapid turnover, in the gastrointestinal mucosa. Histamine acts directly on muscle in the gut wall, not via the enteric neural plexus. Although many gastrointestinal tissues contract in response to histamine, rat ileum and the body of the human stomach are almost unaffected and human colon may relax (63). Histamine may stimulate

excitatory and inhibitory receptors on muscle: the H_1 receptors stimulate and the H_2 receptors inhibit the smooth muscle.

Specific H_1 and H_2 histamine receptors have been identified in the gallbladder wall in primates (64), including humans (65) and in the canine cystic duct (5), but their function is unclear. Increased gallbladder pressure in response to histamine has been demonstrated in several species (64-67); atropine has no effect on this response (64,67), but H_1 receptor blockade diminishes it (64). A similarly increased contractile response of the gallbladder to histamine has been recorded, in the presence and absence of gallstones, in guinea-pigs that had been fed a cholesterol-rich diet (68). Lennon (69), who recently showed dose-related contractions of human gallbladder to histamine in vitro, found that the degree of sensitivity varied with the severity of inflammation in the tissues (69). The predominant effect of histamine on canine cystic duct is to increase resistance to flow and studies in vitro have confirmed that it contracts the duct (5). In health this apparently paradoxical cystic-duct response might be explained by the gallbladder's greater sensitivity to histamine, resulting in contraction of the gallbladder before the onset of change in resistance in the cystic duct. Thus, as with CCK (62), there is no obstruction to the outflow of bile from the gallbladder.

These observations, while illustrating the complexity of the regulation of gallbladder tone and the part histamine may play in it, fail to take into account the possibility that histamine may be released when the wall of the gallbladder or cystic duct is inflamed. This could modify the contractile responses in the cystic duct, as it does in the gallbladder, in cholecystitis (69).

Bradykinin

This naturally active polypeptide can be enzymically released in plasma from inactive precursors (kininogens) by kininogenase or kallikrein activity. The gastrointestinal tract contains kallikreins (63) but no bradykinin precursor, which is said to exist in only the blood and tissue fluid. It is not known whether bradykinin is normally formed within the gut wall and can affect the muscle. However, the presence of bile salts in the colon causes a strong motor response (70); this may be mediated by activation of a kallikrein-like enzyme (71), and Frankish et al. have shown that bile salts in the rat duodenum release and activate kallikreins (72).

Kinin-forming and inactivating activities have been found in bile and gallbladder homogenates (73), and free kinins have been identified in the biliary tract (73). Bradykinin which has been identified as a potent relaxant of the proximal segment of isolated rat duodenum (74,75), contracted isolated rabbit gallbladder (76) and stopped flow through the choledochoduodenal junction in anaesthetized cats (77). Bradykinin also produced dose-related contractions of isolated human gallbladders (69); its sensitivity was not affected by the degree of inflammation (69).

Recent reports indicate that active peptides, in addition to their direct effect on target organs, can modify the effects of other agonists in various tissues (78-80), and that bradykinin modifies the effect of CCK on guinea-pig gallbladder (81). A search of the literature has revealed no reports of bradykinin effects on the cystic duct.

Although no physiological role for bradykinin in motility has been established, it seems likely that inflammation of the gut wall triggers the release of bradykinin; this could affect muscle contractility in that area. Bradykinin almost certainly contributes to the control of blood flow in certain tissues; if this is so in the gut, it might influence cystic-duct motility by altering the blood flow locally.

CYSTIC-DUCT DISEASE

1. In Association with Cholecystitis

Little is known about the clinical association and relationship between cystic-duct disease and cholecystitis. Though the proximal portion of the cystic duct is invariably excised at cholecystectomy, it is rarely examined histologically. The only detailed study that has correlated the pathological abnormalities of the two organs was by Feldman *et al* (82), who studied 150 gallbladders that had been removed together with a segment of the cystic duct.

Some evidence of cystic duct disease was observed in all but nine cases. It was further noted that in general the cystic duct was less inflamed than was the gallbladder. In 56% of cases the cystic-duct lumen was partly or fully occluded. A prior causal relationship of cystic-duct disease with disease of the gallbladder could not be demonstrated. However, experimental studies have shown that partial obstruction to the outflow of bile results in stone formation in the gallbladder (17). Experimental stenosis of the cystic duct in both dogs and rabbits led to formation of pigment-cholesterol stones in 60% of cases (17). There was no bacterial inflammation associated.

The necropsy specimens of human gallbladders with gallstones were examined in one study (17) and 50% had some degree of associated obstruction of the cystic duct. The above studies indicate that stones can form in the gallbladder as a result of stasis alone, and metabolic and bacterial factors need not be implicated in their genesis. The question of whether stasis, as the result of anatomical or functional abnormalities of the cystic duct, is responsible for gallstone formation is still, however, unanswered.

2. Cystic-duct Syndrome

In this condition, the cystic duct is partly obstructed but there are no calculi in the duct or gallbladder. The patients present with histories of typical biliary tract disease and routine investigations including cholecystography are normal. However, more detailed radiological investigation reveals impaired gallbladder evacuation, which at subsequent cholecystectomy, is shown to have been caused by partial cystic duct obstruction (83). This is usually due to either stenosis or kinking of the duct. Histological examination in most cases reveals thickening and fibrosis in its wall (83-85). In McFarland's series (85), nine of ten cases examined had associated chronic cholecystitis. Miesz (84) noted viscous bile in all his cases but made no mention of the gallbladder histology [increased mucus production is a common cause of increased bile viscosity and is now regarded as a feature of pre-gallstone disease (86)]. Another finding in McFarland's and Miesz's studies was that the vast majority of the patients were women of child-bearing age. This is relevant in the light of recent studies that have shown impaired or slow gallbladder emptying in

pregnancy due to the effects of ovarian steroid hormones on gallbladder contractility (87-89). If this is so, there may be more than one cause for this syndrome, or mechanical and/or functional abnormalities are involved. Cholecystectomy relieves the symptoms in the vast majority of cases (83-85).

3. Functional Biliary Disease

Westphal (90) first introduced the term biliary dyskinesia to explain biliary-tract symptoms not associated with pathological abnormalities in either the cystic duct or the gallbladder. Many authors have since attempted to define this entity in terms of a functional disorder of the gallbladder, cystic duct, or sphincter of Oddi. It may be similar to the cystic-duct syndrome, not only in clinical presentation but also in its etiology. In two recently reported series (91,92), all but one of the patients (total, 36) were female. Pre-operatively, in 75% of cases the administration of CCK elicited colicky gallbladder pain and dyspepsia identical to the patients' previous symptoms. In one series, failure of normal gallbladder contraction after cholecystokinin cholecystography occurred in 11 of the patients tested (92). Caroli (93) has shown that lack of contraction in response to cholecystokinin may be due to hormonal imbalance between this substance and anticholecystokinin. At operation in the above series (91,92) the only abnormal feature noted was narrowness of the cystic duct (histological appearances were not mentioned) in many cases; 33% of the gallbladders were normal histologically, and the others had only minimal inflammation. Thirty four of the 36 patients had complete relief of their symptoms at a mean

follow-up of 2 years.It seems that primary cystic-duct disease, if it exists at all, is due to a mechanical or a functional abnormality or both.

Another recent theory to explain biliary dyskinesia relates to an abnormality in the activity of the sphincter of Oddi. There is circumstantial evidence to suggest that, normally, CCK acts on the sphincter to produce a decrease in resistance to flow across it (54). This effect may be produced by stimulation of nonadrenergic, noncholinergic inhibitory nerves (54). This neural inhibitory action of CCK overrides the direct stimulatory effect of CCK on the smooth muscle of the sphincter, which has been reported (94). Imbalance between the dominant inhibitory effect of CCK on this sphincter and CCK direct smooth-muscle stimulatory action may lead to inappropriate spasm of the sphincter instead of the expected relaxation. Such an abnormality in the motility of the sphincter has been proposed as an explanation for biliary dyskinesia (95).

MUSCLE MORPHOLOGY

Unlike the rest of the gut, which has well-defined circular and longitudinal muscle coats in addition to the muscularis mucosae, the gallbladder has only one (in many cases, thin) layer of muscle, which is continuous with that of the cystic duct. This layer resembles the intestinal muscularis mucosae. Because so little is known of muscle morphology in calculous cholecystitis, it is difficult to correlate morphological changes with motility. The muscle layer seems to be composed of a three-dimensional mesh of interconnecting muscle bundles,

apparently arranged in spirals (29). Changes in the thickness of the muscle layer, which decreases gradually from the fundus of the gallbladder to the distal end of the cystic duct (29), and in its state of health could alter gallbladder and cystic-duct motility. The sensitivity of the muscle to neural, hormonal or local stimuli may change when it becomes inflamed. Conversely inflammation *per se* may affect the ability of the muscle to respond to these stimuli (38). If the inflammatory changes induced by cholelithiasis vary within the gallbladder and cystic duct and similar variations occur in the muscle layer, these differences could affect extrahepatic biliary motility.

THE PRESENT STUDY

The gallbladder neck and the cystic duct are of particular interest from the standpoint of functional morphology (96). The importance of these areas in the integrated functioning of the extrahepatic biliary tract is indirectly confirmed by the very rich innervation present (30,31). The controversy about the presence or absence of an anatomical or physiological sphincter in the neck of the gallbladder or at the junction of the neck and the cystic duct is still unresolved.

The aims of the study were:

1. To examine the body and neck of the gallbladder and the cystic duct in healthy dogs and in humans with cholecystitis in order to see if a transition or change occurs in the histological features in the three areas. The pathological incidence of cystic duct disease in association with cholecystitis were also be analyzed.
2. To investigate the nature and thickness of the smooth muscle layer in the gallbladder and the cystic duct, particularly in the neck region of the gallbladder.
3. To study the spontaneous and stimulated activity of the cystic duct in vitro, and correlate this with gallbladder activity.

MATERIALS AND METHODS

Gallbladders from 107 patients and cystic ducts from 21 were studied. The cholecystectomies were performed in the University of Alberta Hospitals and the Charles Camsell General Hospital, Edmonton. The cases were unselected except in relation to scheduling the laboratory experiments.

In addition, 15 canine gallbladders and cystic ducts were studied (see Non-Diseased Tissues), as it was not possible to obtain normal human tissue.

Collection of Human Gallbladders and Cystic Ducts

The gallbladder and the proximal part of the cystic duct (in 21 cases) were collected as soon as they were removed. The ischaemic period (i.e., time taken from ligation of the cystic artery to delivery of the gallbladder) was no longer than 20 minutes. The organ was placed in oxygenated Krebs' solution immediately, examined by the hospital pathologist, and opened along its longitudinal axis. Two adjacent rectangular pieces, 4X1.5 cm., were excised from the anterior wall of the body of each organ. A portion from the neck in each organ and the cystic duct in 21 cases was also excised. These were transported in Krebs' solution to the laboratory.

Non-Diseased Tissues

Gallbladders and cystic ducts from 15 apparently healthy mongrel dogs were studied. The dogs, weighing 10 to 20 kg, were of both

sexes. They had been rendered free of intestinal parasites, and inoculated against rabies and distemper, for surgical procedures by other investigators (who took over as soon as the gallbladder had been removed). Thus, selection was uncontrolled except in relation to scheduling the laboratory experiments.

The dogs were anaesthetized with sodium pentobarbital (300 mg/kg body weight) injected iv, and an endotracheal tube was inserted to maintain an airway. They were allowed to breathe spontaneously (room air) during the surgical procedure. The gallbladder and cystic duct was removed through a midline incision and immediately washed free of bile in Krebs' solution. Tissue was excised from the wall of the body of the gallbladder and the cystic duct, as with the human tissue.

Preparation of Tissue

One piece of tissue from the body and neck of each gallbladder was processed for histology and measurement of the muscle and the other pieces were cut into longitudinal strips, 2X10 mm. One piece of tissue from the cystic duct (n=16) was also processed for histology and measurement of the thickness of the muscle layer and the other piece was cut into either two or three longitudinal strips, 2X10 mm. Contractility studies of the strips, with standard organ-bath techniques, were started within 1 h of the cholecystectomy.

Histology

Tissue for light microscopy was rinsed in Krebs' solution, fixed for 24 h in 10% neutral buffered formalin and embedded in paraffin (Tissue-prep; Fisher Scientific Ltd., Edmonton) at 56.5°C. Transverse

sections, cut at 8 to 10 μm, were floated onto albumen-coated slides (up to 5, in sequence, per slide). All were stained with haematoxylin and eosin. Coverslips were applied with Histoclad (Clay-Adams) mounting medium.

The slides were coded, mixed, and examined without knowledge of the results of motility studies. On transverse sections, a grading was assigned to each component of the inflammatory infiltrate, extravasation of red cells, and oedema and fibrosis in the mucosa and lamina propria, muscle layer, and areolar layer. These were scored 0, normal; 1, definite but mild histopathological abnormality; and 2, advanced histopathological abnormality. A score of 2 was added if the mucosa was absent because of disease (Fig. 1).

The tissue on each slide was also classified as typical of mild chronic, advanced chronic, or acute cholecystitis, without reference to the total score. Finally, each score was totalled and the Hospital's pathological classification was documented. The same scoring and classification system was used on the cystic duct specimens.

Measurement of Muscle Thickness

The muscle thickness was measured on each transverse section; 10 duplicate coded slides were added, to assess the accuracy of the technique. Measurement was by a blind random-sampling method. Starting from one edge of a section, muscle thickness was measured with an eyepiece micrometer (10X objective). Using a 1 mm stage-mounted scale on both the X and Y axes, full-wall muscle thickness was measured at 3 mm intervals; measurements were made in at least two (usually three) sections on each slide, starting from the opposite ends of each

TRANSVERSE SECTION

Code no._____

Date _____

Score	Overall impression
Mucosa (incl.lamina propria)	
thickness _____	
cholesterolosis _____	Normal _____
ulceration _____	
infiltrate: lymphocytic ____	Cholesterolosis _____
neutrophilic ____	
eosinophilic ____	
RBCs _____	Chronic: mild _____
edema _____	moderate _____
Muscle layer	severe _____
thickness _____	
connective tissue _____	Acute _____
infiltrate: lymphocytic _____	
neutrophilic_____	Mucocoele _____
eosinophilic_____	
RBCs _____	
fibrosis _____	
edema _____	
Areolar layer	
infiltrate: lymphocytic_____	
neutrophilic_____	
eosinophilic_____	
RBCs _____	
fibrosis _____	
edema _____	

FIGURE 1

Histological Scoring Sheet

subsequent section. Only the thickness of the muscle bundles was recorded (interspersed fibrous connective tissue was not included). Finally, the number of units of muscle per full wall thickness was totalled and was converted to micrometres, the mean thickness of specimens on each slide was computed, and the standard deviation was calculated.

Motility Studies

The Krebs' solution had the following composition (mM): NaCl, 116; KCl, 5.4; $CaCl_2$, 1.2; NaH_2PO_4, 1.2, $NaHCO_3$, 22; and D-glucose, 11.2. The solution (pH 7.4) was made up in distilled de-ionized water, equilibrated with 95% O_2 and 5% CO_2, and maintained at 37°C.

Drugs used and their sources were as follows: Acetylcholine chloride, histamine diphosphate, bradykinin triacetate, (Sigma Chemical Co.); CCK octapeptide (CCK-OP; Squibb Chemical Co.).

Stock solutions (1-10 mg/ml) of all drugs were prepared and were diluted with distilled water before each experiment.

Tissue Preparations

Gallbladder and cystic duct strips were mounted along their longitudinal axis in 5 ml organ baths. One end of each strip was attached with a 000 silk ligature to a platinum hook electrode at the bottom of the bath, and the other end was attached with a similar ligature to an isometric tension transducer. The bath contained Krebs' solution pH 7.4, maintained at 37°C and continuously gassed with 95% O_2 and 5% CO_2. Isometric contractions in response to the application of test drugs were measured with force-displacement transducers (FT03C;

Grass Instrument Co., Quincy, Mass.) connected to a Beckman polygraph recorder. Responses were taken as the change in grams tension from the baseline. The molar concentration of the drug in the organ bath was recorded as the concentration at which the response occurred.

The strips were adjusted to an initial tension of 500 mg and, during 45 min equilibration, they were washed continuously with Krebs' solution at 5 ml/min. In all but a few strips the basal tension remained stable thereafter; in those in which it rose spontaneously tension was readjusted to 500 mg manually,. Each strip was exposed to a maximally effective concentration of acetylcholine (1 mM), as a test of viability and as an index of the maximal responsiveness of each tissue. Following this stimulation, 15 min was allowed before further agents were tested.

Application of Test Drugs

Each agonist drug was tested on at least two strips from each of the human and canine gallbladders and at least one from each cystic duct used in particular experiments, and non-cumulative concentration-response curves were constructed. Strips were exposed to increasing concentrations of the drug until the maximal response was obtained. The drug was washed from the bath by overflow of fresh Krebs' solution, before the next concentration was added. For each drug, the same waiting period was allowed between additions; this ranged from 5 to 20 min, because one drug (histamine) caused tachyphylaxis, necessitating repeated washing, and with CCK-OP the return to baseline tension usually was delayed.

Analysis of Data

For each drug tested, contractility responses were expressed as a percentage of the maximal contraction (100%) of the strips. These values were plotted on a concentration-response curve.

The ED_{50} value (agonist concentration that produced half the maximal response) was calculated for each concentration-effect curve for each gallbladder strip.

Mean values, standard deviation (SD), and standard error of mean (SEM) were calculated for each group. Significant levels for the difference between groups were estimated using Student's paired, and in relevant cases, unpaired *t*-test. The difference between groups was judged to be significant when $P<0.05$.

In the Results section, values are given as the mean ± the standard error of mean (SEM). Non-significant differences are stated as NS.

RESULTS

There were 86 females and 21 males in this study, giving a female:male ratio of 4:1. Their mean age was 49 years (48 years for females and 57 years for males). Eighy-four of the 107 patients had symptoms suggestive of gallbladder disease at the time of their hospital admission, which was elective for 76 and emergency for 31.

Calculi were present in 99 gallbladders, and in three of the other eight there was reason to believe calculi had been present. The calculi were solitary in 20 cases and multiple in the other 79. The gallbladder was obstructed by a calculus impacted in its neck or in the cystic duct in 43 cases, including 11 in which the calculus was solitary.

HISTOLOGY

Use of the histological scoring and classification system identified three types of inflammation: mild chronic, advanced chronic (moderate or severe), and acute cholecystitis (Fig. 1). Comparison of the scores allocated to the gallbladder body and neck in each group showed no significant intragroup differences (Table 1). The histological scores for the 16 cystic ducts examined were similar to those allocated to the corresponding gallbladders (Table 2).

Gallbladder

The same abnormalities, including cholesterolosis, were noted in the gallbladder body and neck, and the distribution of fibrosis, edema, and red-cell extravasation was similar in both regions.

TABLE 1

Histological Scores for Diseased Human Gallbladders

	Histological Score	
Cholecystitis Group	Body	Neck
Mild Chronic	5.3 ± 0.3 (n=29)	5.2 ± 0.4 (n=25)
Advanced Chronic	8.5 ± 0.3 (n=51)	7.3 ± 0.4 (n=48)
Acute	12.5 ± 0.6 (n=18)	11.6 ± 0.6 (n=15)

TABLE 2

Comparison of Histological Scores for Human Gallbladders with their Corresponding Cystic Ducts in Chronic Cholecystitis

Group	Histological Score
Gallbladder Body (n=16)	6.25 ± 0.54
Cystic Duct (n=16)	5.75 ± 0.59

In both mild and advanced chronic cholecystitis, mucosal hyperplasia and polyposis were prominent features (Fig. 2 A,B), especially in the neck region, where folds were commonly taller and more numerous. Cholesterolosis was clearly evident in both regions in several specimens. About 20% of the neck sections examined contained scattered mucus glands deep to the mucosal layer (Fig. 2 A,B,); they were distinct from Rokitansky-Aschoff sinuses, and their cells were usually large and lightly stained. The inflammatory infiltrate, which was predominantly lymphocytic, was evident in the mucosa, and usually just deep to the muscle layer. In the mildly inflamed gallbladders, the areolar layer was the thickest; whereas those evidencing progressive disease had undergone hypertrophic change, with increasing relative thickness of both the mucosal and the muscle layers. In advanced chronic cholecystitis, in many cases the muscle layer was the wall's thickest layer and in most was thinner in the neck than in the body.

In acute cholecystitis, the epithelial changes were closely associated with the nature of the inflammation. There was extensive mucosal ulceration: in many cases the epithelium was completely replaced by regenerating or metaplastic epithelium (low, cuboidal, with central nuclei). In the 'acute' group the wall was commonly replaced by scar tissue. In some, however, though the entire body was fibrosed the neck contained muscle fibers. In some cases, large polyps were noted in the neck but none in the body.

Cystic Duct

Many of the histological features noted in the gallbladders with chronic cholecystitis, including mucosal polyposis, were seen in the

FIGURE 2

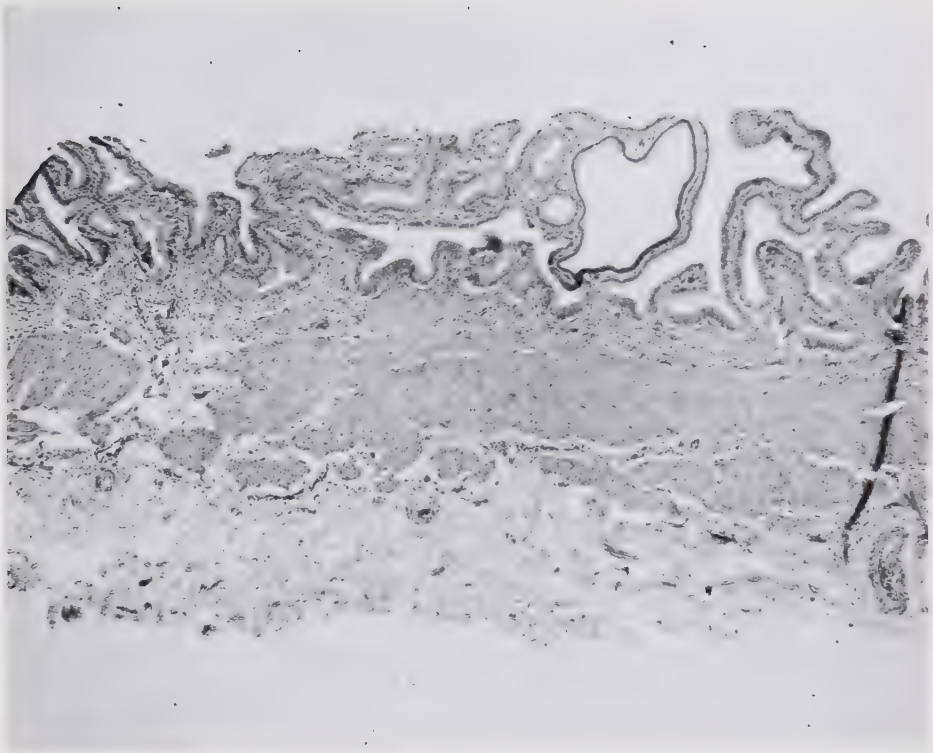
Characteristic Appearance of Human Gallbladder Body and Neck, and Cystic Duct, in Chronic Cholecystitis

Transverse sections from one gallbladder. The muscle layer is interspersed with layers of loose connective tissue. (Staining with hematoxylin and eosin. Magnification, X 35.)

FIGURE 2A. Gallbladder body. The mucosal and muscle layers are thickened. Mucosal inflammatory infiltrate is slight. Topography of the muscle layers shows longitudinal interspersed with circular muscle bundles.

FIGURE 2B. Gallbladder neck. Mucosal hyperplasia is evident. The muscle layer is thinned and there are wide gaps between some of the muscle bundles. Mucus glands are present in the submucosa and muscle layer.

Figure 2A



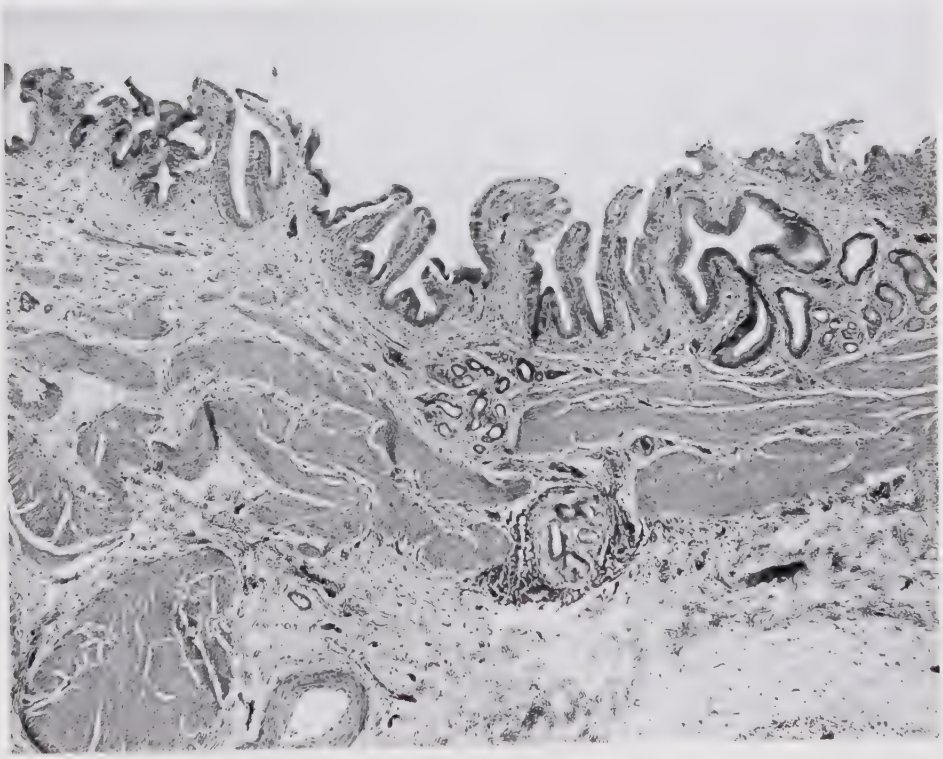


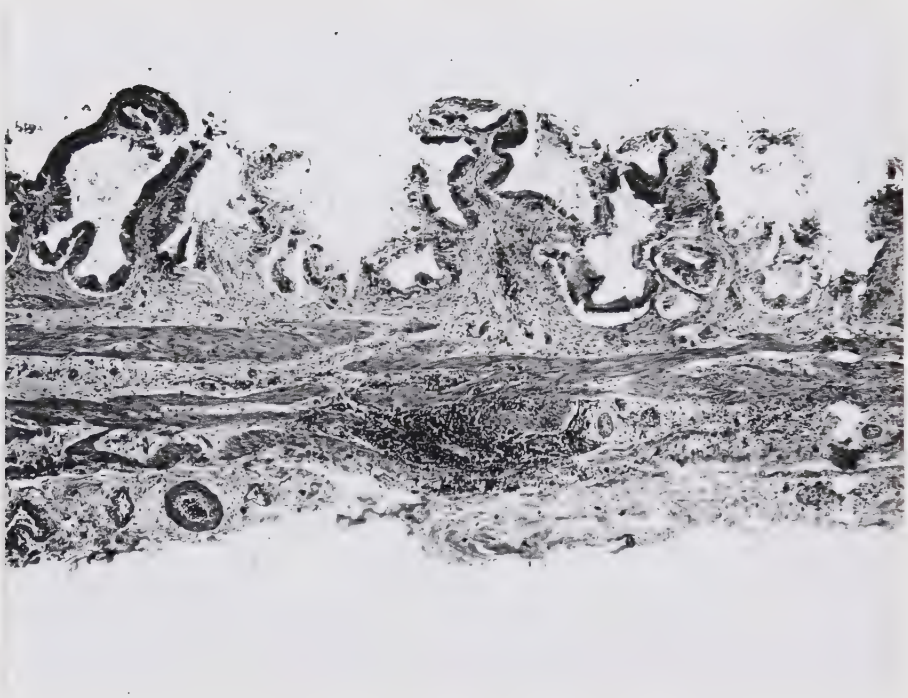
Figure 2B

FIGURE 2

(Continued)

FIGURE 2C. Cystic duct. The mucosal layer is thickened and foci of ulceration are evident. There is a moderate lymphocytic infiltrate throughout all three layers. The muscle layer is thin, and the muscle bundles are separated by interspersed connective tissue.

Figure 2C



corresponding cystic ducts (Fig. 2C); a notable exception was focal mucosal ulceration which was present in the duct of several specimens. In 6 of the 16 specimens examined, mucus glands were found in the mucosal layer; some of these contained an accumulation of 'milky' mucus in apical areas of the epithelium and in the villous polyps. The muscle layer was thin in all, and the muscle bundles were widely separated by connective tissue in many (Fig. 2C). One of the most notable histological features was fibrosis, commonly extending through all three layers; it was present in more than half the cystic ducts examined

CANINE GALLBLADDERS AND CYSTIC DUCTS

The only notable feature detected was a lymphocytic mucosal infiltrate in two of the seven gallbladders examined. Mucus glands were found in the body of three gallbladders and in the cystic ducts. In both gallbladder and cystic duct, accumulations of mucus were found in the apical epithelium and the muscle layer was thin (Fig. 3 A,B).

MORPHOLOGY AND THICKNESS OF GALLBLADDER AND CYSTIC-DUCT MUSCLE

The morphology was similar in the diseased human and normal canine gallbladder and cystic duct. The muscle layer commenced just deep to the base of the mucosal folds. On transverse section (Figures 2,3), it appeared to be composed of longitudinal, circular, and oblique muscle bundles; longitudinal muscle bundles were more numerous in the canine gallbladders (Fig. 3A).

FIGURE 3

Characteristic Appearance of Normal Canine Gallbladder Body and Cystic Duct

Transverse sections. Staining with hematoxylin and eosin; Magnification, X 35.

FIGURE 3A. Gallbladder body. There is some stunting of mucosal folds. The muscle layer is tightly packed with mainly longitudinal muscle bundles. There is a mild lymphocytic infiltrate in the mucosal and areolar layers.

FIGURE 3B. Cystic duct. The mucosa bears small polyps and contains a lymphoid follicle, and the mucosal epithelium contains accumulations of mucus in its apical areas. The muscle layer is very thin.

Figure 3A

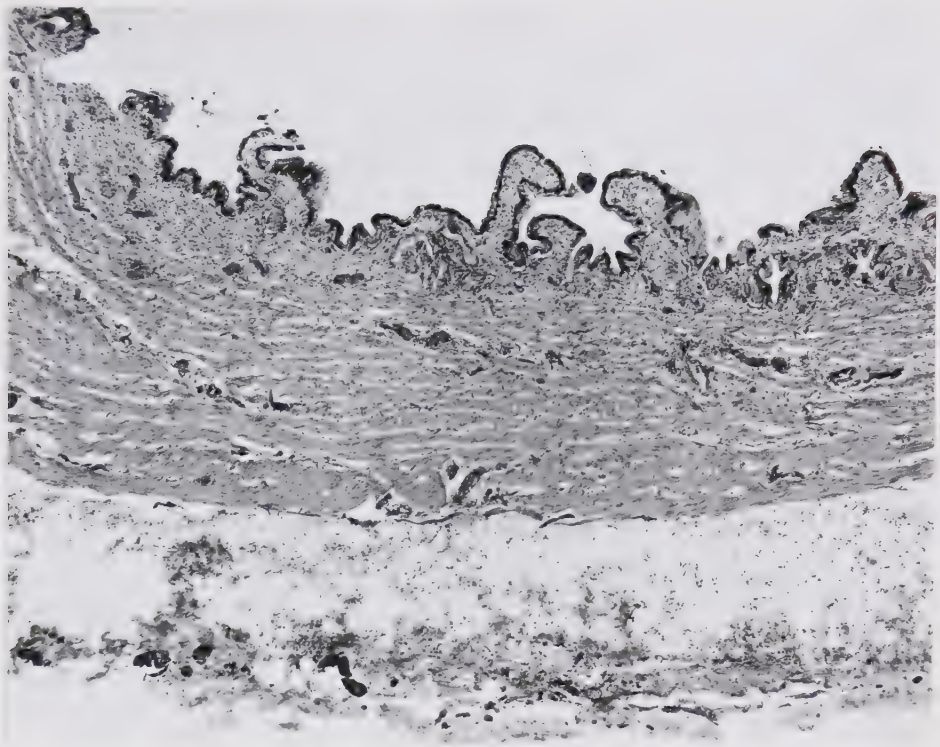


Figure 3B

TABLE 3

Muscle Thickness in Human Gallbladder
Body and Neck

Cholecystitis Group	Thickness ($\bar{x} \pm$ SEM, μm)	
	Body	Neck
Mild chronic	300.0±18.0 (n=29)	263.0±24.0 (n=21)
Advanced chronic	466.2±23.3 (n=50)	331.0±18.0 (n=48)
Acute	245.0±46.3 (n=17)	310.0±35.0 (n=12)

TABLE 4

Comparison of Muscle Thickness in Human
Gallbladders with their Corresponding Cystic Ducts

Group	Thickness ($\bar{x} \pm$ SEM, μm)
1. Gallbladder Body (n=7)	423.0 ± 21.7
2. Gallbladder Neck (n=7)	300.0 ± 24.5
3. Cystic Duct (n=7)	109.0 ± 18.0

1 vs 2 $P<0.05$ 1 vs 3 $P<0.05$ 2 vs 3 $P<0.05$

TABLE 5

Muscle Thickness in Canine
Gallbladder Body and Cystic Duct

Group	Thickness ($\bar{x} \pm$ SEM μm)
1. Gallbladder (n=7)	287.3 ± 33.7
2. Cystic Duct (n=5)	44.0 ± 12.0

1 vs 2 $P<0.05$

The mean thickness of the muscle layer was less in the neck than in the body of gallbladders, the difference was significant in mild and advanced chronic cholecystitis. but not in acute cholecystitis (Table 3). In both human and canine specimens, the mean thickness of the muscle layer was significantly less in the cystic duct than in the gallbladder (Table 4,5).

MOTILITY OF DISEASED HUMAN GALLBLADDERS AND CYSTIC DUCTS

Only gallbladders with chronic cholecystitis and their corresponding cystic ducts were studied. It was not possible to harvest cystic ducts at cholecystectomy in cases with acute cholecystitis because of technical difficulties.

SPONTANEOUS ACTIVITY

Spontaneous contractile activity occurred in 42 of 72 human gallbladder strips. When it occurred in strips from the gallbladder body it was also present in strips from the neck. It was present in 22 of 53 cystic duct strips. It was detectable soon after the tissues were set up, and was well established when experiments were begun 60 min. later. The activity varied widely in both frequency and amplitude, but once these characteristics had been established for a tissue they were maintained for the duration of the experiment. The frequency of spontaneous activity was similar in strips from all three regions. It varied from 2 to 5 per minute. The amplitude was the same in strips from the body and neck of the gallbladder but was lower in the cystic

duct strips. It varied in all three regions from about 10% to 70% of the maximal contraction to acetylcholine.

This activity rarely masked the drug-induced response of a preparation. In most cases, the addition of a drug induced a tetanic-like contractile response and reduced or abolished the spontaneous activity. At near-maximal and maximally effective concentrations of drug, there was usually no trace of spontaneous activity associated with the contraction to the drug.

DRUG-INDUCED RESPONSES

The tone of the strips was adjusted initially to 500 mg. It was maintained at this level during the experiments. The onset and rate of rise of the responses to the various agonists used was similar in strips from the body and the neck of the gallbladder. The cystic duct responses to acetylcholine, histamine and CCK-OP reached a peak height more slowly, and were more sustained than those from either the body or neck of the gallbladder. There was very little difference in the response pattern of the strips to bradykinin in any of the 3 regions.

Acetylcholine

Acetylcholine induced contractile responses in all the gallbladder and cystic duct strips tested. The threshold response occurred between 10^{-7}M and 10^{-6}M concentration in both groups. The contractile responses were concentration-dependent. There was no significant difference in sensitivity between the gallbladder and the cystic duct strips (Fig. 4) [ED_{50}(μM) 16.9±4.1 and 9.6±0.9 respectively]. However, the mean maximal

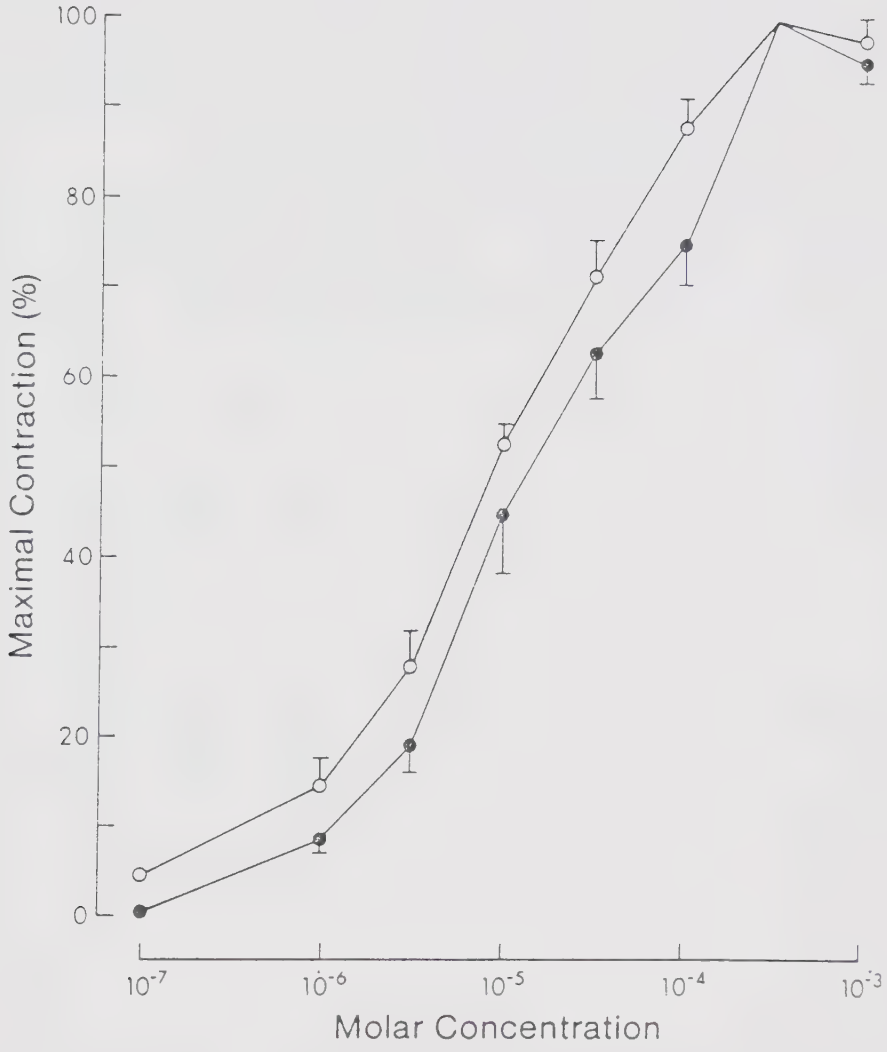


FIGURE 4

Concentration-Response Curves to Acetylcholine in Untreated Strips of Human Gallbladder Body and Cystic Duct

Acetylcholine-induced contractile responses of strips of gallbladder body (●—●, n=4), cystic duct (○—○, n=4). Results (mean ± SEM) are expressed as percentages of the maximal response.

response was significantly greater in the gallbladder strips (0.79±0.09g vs 0.28±0.02g).

Cholecystokinin Octapeptide

CCK-OP consistently produced concentration-dependent contractions in strips of human gallbladder and cystic duct. Strips from 11 gallbladders and cystic ducts with chronic cholecystitis were studied. In a further two cystic ducts, the strips did not respond to CCK-OP (10^{-11}M - 3 x 10^{-7}M). The mean sensitivity and maximal response to CCK-OP of gallbladder body and neck strips was the same (Fig. 5) [ED_{50}(nM) 20.7±5.4 and 15.7±3.7; max. response(g); 0.46±0.11 and 0.40±0.08 respectively]. In the cystic duct the sensitivity to CCK-OP was significantly decreased (Fig. 5). The mean ED_{50}(nM) was 63.2±13.4. The apparent lower mean maximal response in the cystic duct strips (0.29±0.07 g) was not significantly different from the gallbladder strips.

Histamine

Histamine (10^{-6}M - 10^{-3}M) contracted gallbladder and cystic duct strips, maximally at about 3 x 10^{-4}M. (This may not have been the true maximal response, as the tissue frequently did not respond reproducibly to higher concentrations). Histamine (10^{-6}M - 10^{-3}M) had no effect on three gallbladders and their cystic ducts but in another 8 it caused concentration-dependent contractions (Fig. 6). There was no significant difference between the gallbladder and cystic duct sensitivity [ED_{50}(μM): 15.1±2.7 and 18.4±4.9 respectively]. Strips from only three

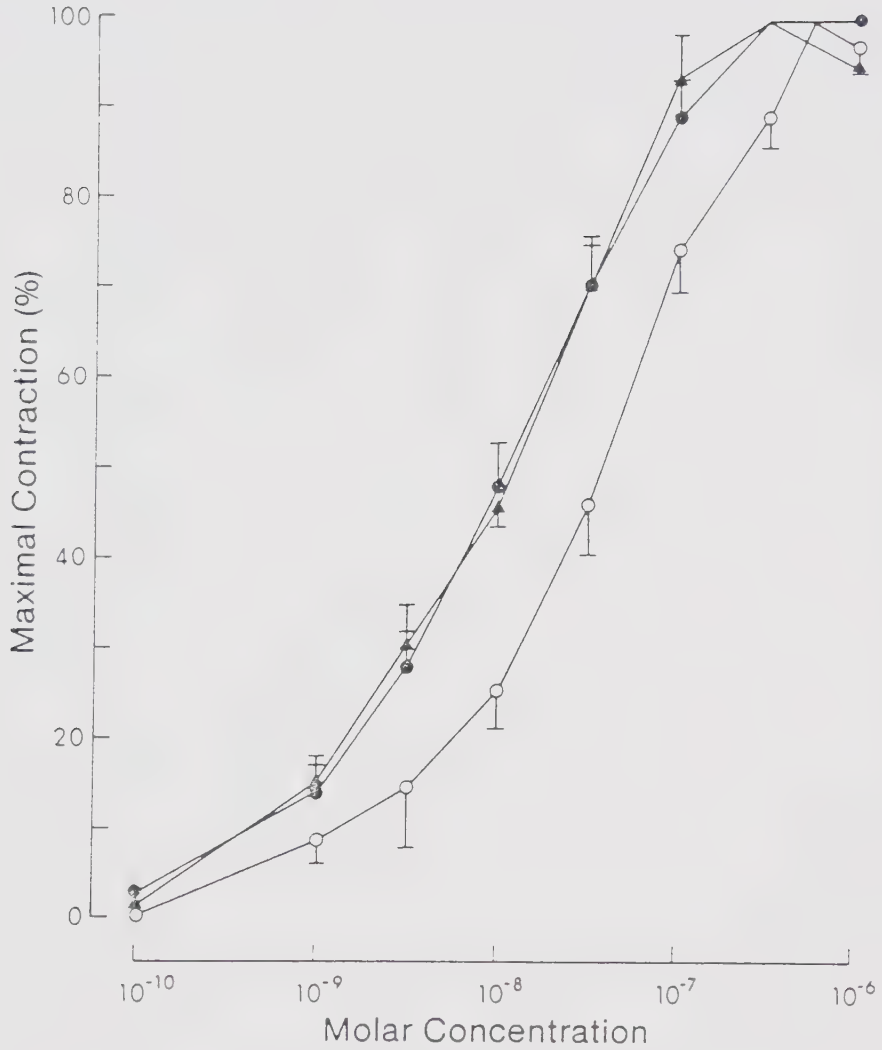


FIGURE 5

Concentration-Response Curves to CCK-OP in Untreated Strips of Human Gallbladder Body, Neck and Cystic Duct.

CCK-OP-induced contractile responses of strips of gallbladder body (●—●, n=10), gallbladder neck (▲—▲, n=5), cystic duct (○—○, n=10).
Results (mean ± SEM) are expressed as percentages of the maximal response.

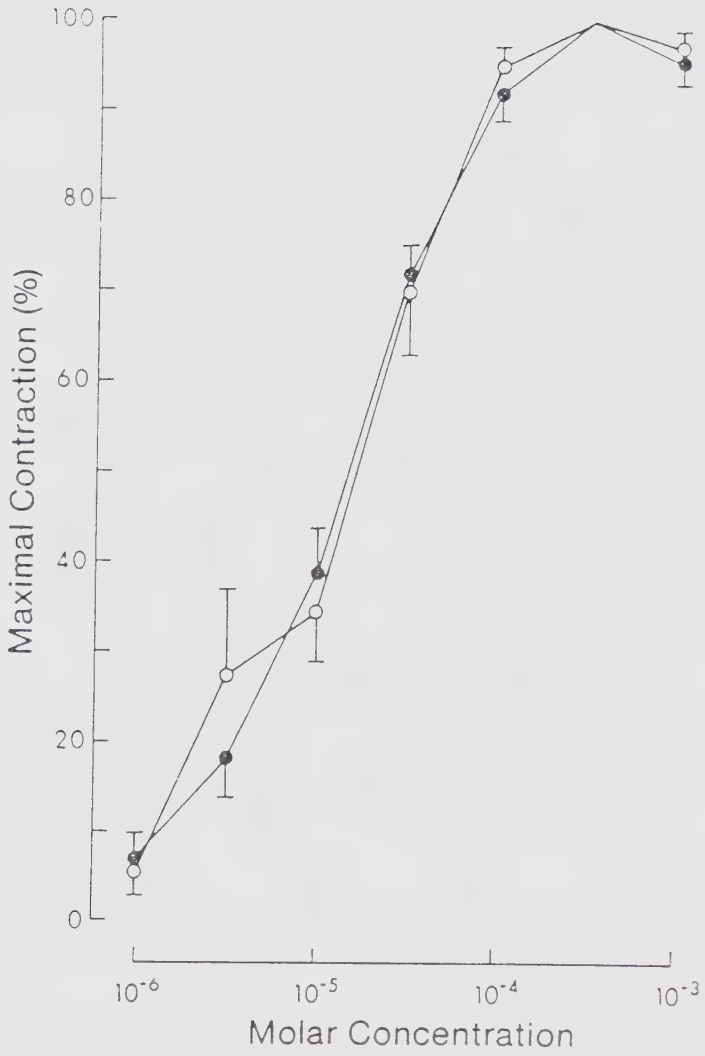


FIGURE 6

Concentration-Response Curves to Histamine in Untreated Strips of Human Gallbladder and Cystic Duct

Histamine-induced contractile responses of strips of gallbladder body (●—●, n=8), cystic duct (o—o, n=8). Results (mean ± SEM) are expressed as percentages of the maximal response.

gallbladder necks were examined and their sensitivity was similar to the other two regions. The mean maximal response was significantly smaller in the cystic duct than in the gallbladder body (0.14±0.05g vs 0.83±0.25g).

Bradykinin

Bradykinin consistently produced contractions of isolated gallbladder and cystic duct strips; on a molar basis, it was about 10 times more active than histamine (Fig. 7). The maximal responses to bradykinin were greater than with any of the other three drugs tested. The responses occurred in the 10^{-8}M to 5 x 10^{-5}M range, maximal at 3 x 10^{-5}M. The sensitivity of the strips from the gallbladder neck and cystic duct was similar (ED_{50}(μM): 0.90±0.27 and 1.08±0.26 [n=9]). The cystic duct and gallbladder neck strips were significantly more sensitive ($P<0.05$) than the corresponding strips from the gallbladder body (ED_{50}μM): 2.04±0.33 [n=9]). The maximal responses were significantly greater in the body than in the neck or cystic duct (1.05±0.19 g; 0.50±0.11 g; 0.34±0.73 g respectively).

MOTILITY OF HEALTHY CANINE GALLBLADDERS AND CYSTIC DUCTS

SPONTANTEOUS ACTIVITY

Spontaneous activity was present in 40 of the 45 gallbladder strips examined and in 23 of the 30 cystic duct strips examined. Usually, it commenced soon after the preparation was mounted and continued throughout the experiment. The degree of this activity varied among the

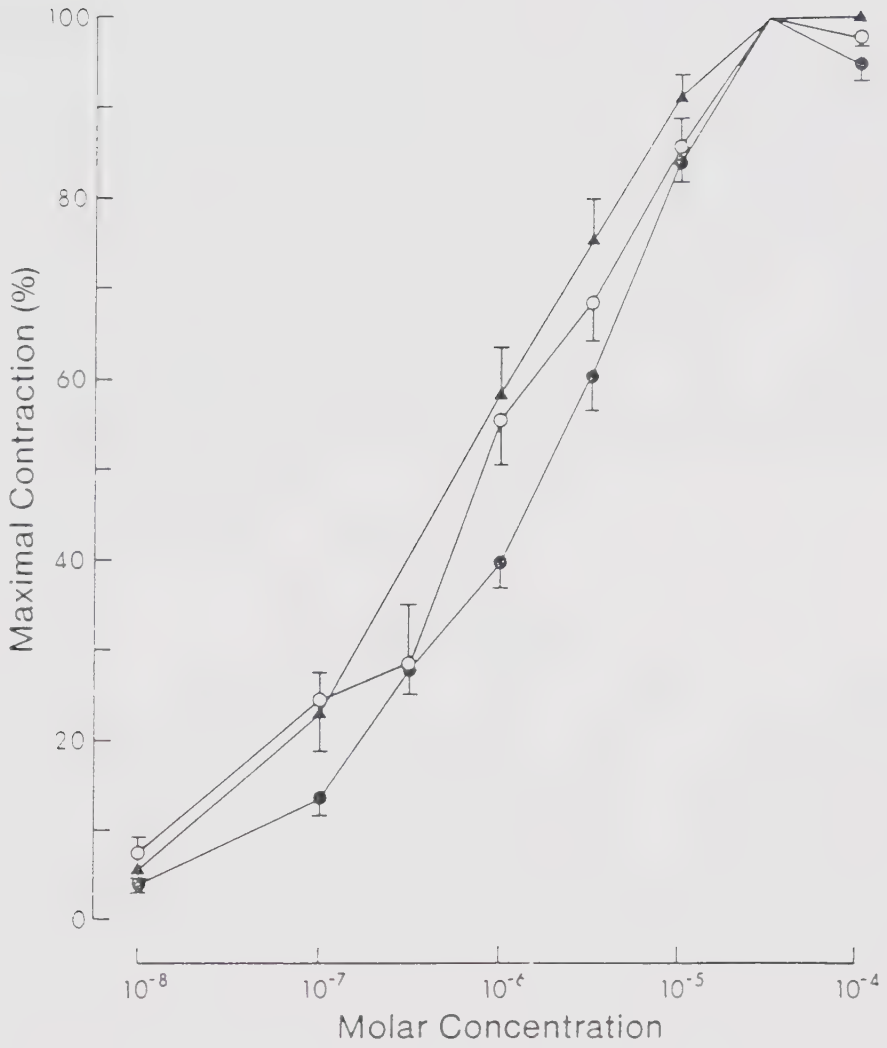


FIGURE 7

Concentration-Response Curves to Bradykinin in Untreated Strips of Human Gallbladder Body, Neck and Cystic Duct.

Bradykinin-induced contractile responses of strips of gallbladder body (●—●, n=9), gallbladder neck (▲—▲, n=9), cystic duct (○—○, n=9).
Results (mean ± SEM) are expressed as percentages of the maximal response.

preparations within each group. In general the frequency was similar in the gallbladder and the cystic duct strips, but the amplitude was lower in the cystic duct preparations.

DRUG-INDUCED RESPONSES

The tone of the strips was adjusted initially to 500 mg. The tone did not rise during equilibration or during the experiments. The cystic duct, in the vast majority of cases, responded as did the human cystic duct to acetylcholine, histamine and CCK-OP with a contraction that reached peak height more slowly, and was more sustained than was observed with the gallbladder responses. This response pattern was less marked with bradykinin.

Acetylcholine

All the gallbladder and cystic duct strips responded to acetylcholine (10^{-3}M). Consistent concentration-dependent contractions occurred in both sets of strips. There was no significant difference in the sensitivity between the two organs (Fig. 8, Table 6). However, the mean maximal responses were greater in the gallbladder strips ($P<0.01$).

Histamine

Histamine produced consistent concentration-dependent contractions in both gallbladder and cystic duct strips. There was no significant difference in the sensitivity between the two groups but the mean maximal responses were greater in the gallbladder strips (Fig. 9, Table 6).

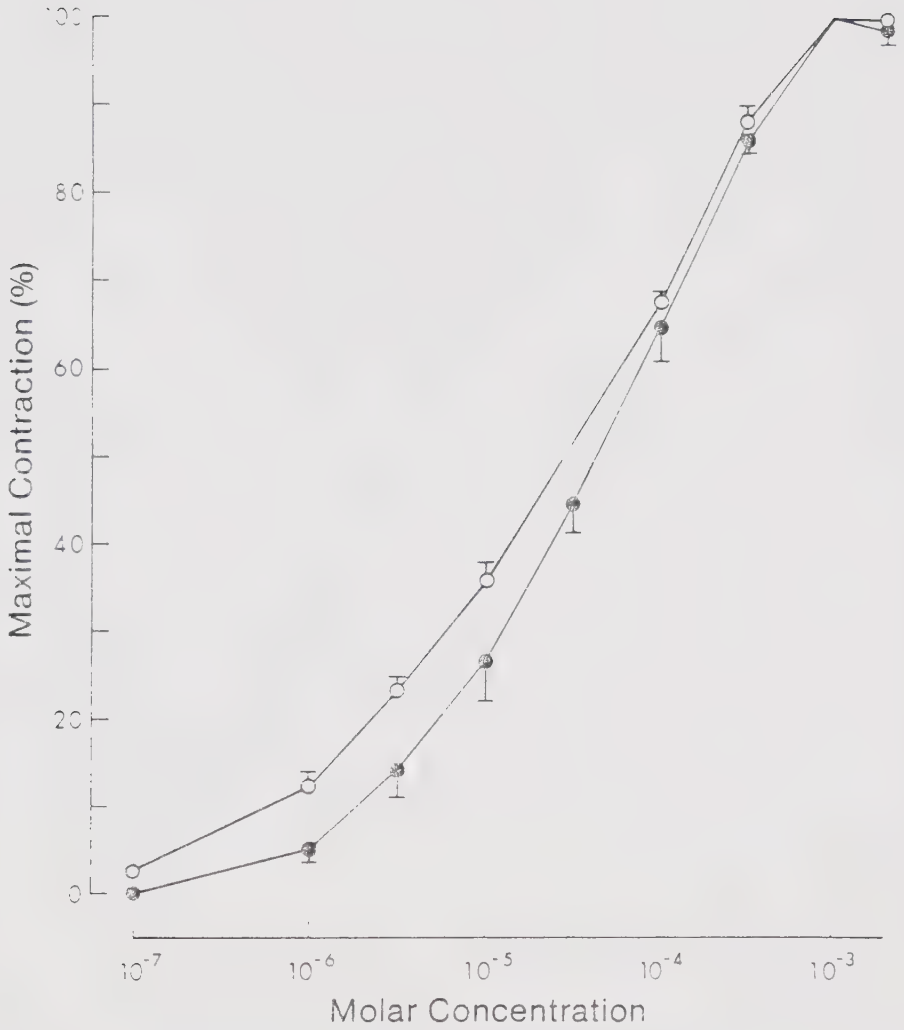


FIGURE 8

Concentration-Response Curves to Acetylcholine in Untreated Strips of Canine Gallbladder and Cystic Duct

Acetylcholine-induced contractile responses of strips of gallbladder body (●—●, n=5), cystic duct (○—○, n=4). Results (mean ± SEM) are expressed in percentages of the maximal response.

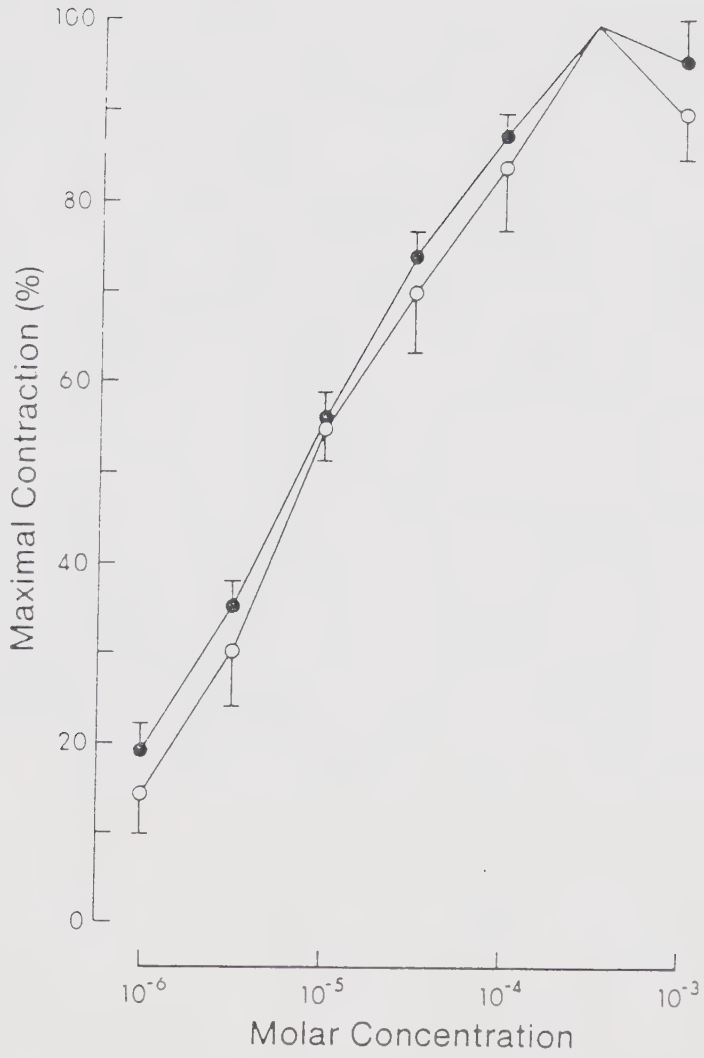


FIGURE 9

Concentration-Response Curves to Histamine in Untreated Strips of Canine Gallbladder Body and Cystic Duct

Histamine-induced contractile responses of strips of gallbladder body (●—●, n=7), cystic duct (o—o, n=8). Results (mean ± SEM) are expressed as percentages of the maximal response.

Bradykinin

Bradykinin produced consistent concentration-dependent contractions in both organs in the concentration range 10^{-8}M to 5 x 10^{-5}M. The sensitivity of the strips to bradykinin were similar in both groups but again the mean maximal response was significantly greater in the gallbladder strips (Fig. 10, Table 6).

Cholecystokinin Octapeptide

The gallbladder and cystic duct strips responded to CCK-OP in a concentration-dependent manner. The ED_{50}(nM) for the gallbladder was 4.3±3.2. Only 4 cystic duct strips from 2 dogs were examined. It was not possible to calculate an ED_{50} in 2 cystic duct strips because the maximal response was not reached at a concentration of 3 x 10^{-7}M CCK-OP. In the other 2 strips from one dog the ED_{50}'s were 10 and 15 nM. The mean ED_{50} in the cystic duct in this dog was approximately 3 times greater than the corresponding ED_{50} for the gallbladder.

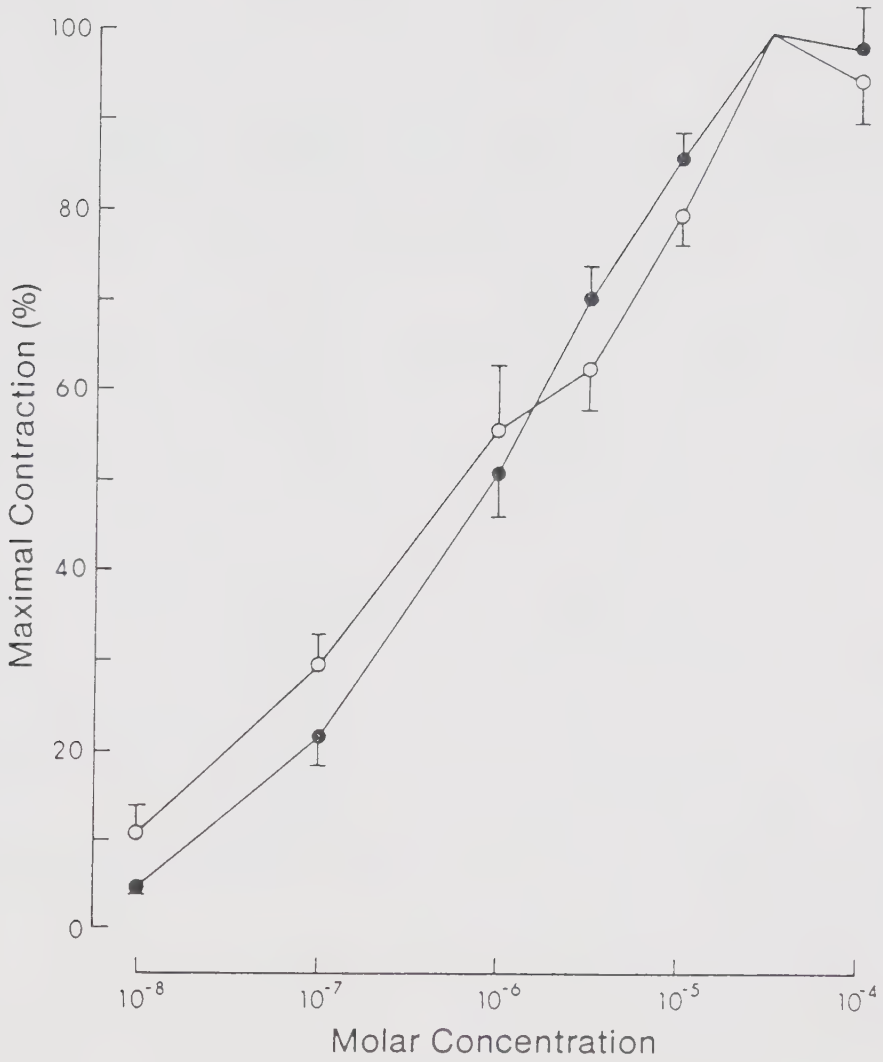


FIGURE 10

Concentration-Response Curves to Bradykinin in Untreated Strips of Canine Gallbladder and Cystic Duct

Bradykinin-induced contractile responses of strips of gallbladder body (●—●, n=5), cystic duct (o—o, n=5).
Results (mean ± SEM) are expressed as percentages of the maximal response.

TABLE 6

RESPONSE OF CANINE GALLBLADDER (GB) AND CYSTIC-DUCT (CD) STRIPS TO ACETYLCHOLINE, HISTAMINE, AND BRADYKININ.

		ED_{50}(μM)	Maximal Response (g)
Acetylcholine	GB (n=5)	43.6 ± 7.5	2.08 ± 0.20
	CD (n=4)	31.8 ± 3.8	0.24 ± 0.04
Histamine	GB (n=7)	7.8 ± 0.8	0.78 ± 0.07
	CD (n=7)	8.0 ± 1.2	0.22 ± 0.07
Bradykinin	GB (n=5)	0.99 ± 0.17	1.29 ± 0.11
	CD (n=5)	0.98 ± 0.38	0.20 ± 0.06

DISCUSSION

HISTOPATHOLOGY

Gallstones develop within the lumen (i.e, not in the wall) of the gallbladder and can float freely within the organ without producing any symptoms, pathological changes, or disorders of function (97). However, small stones or pieces of stones can pass into and through the cystic duct into the common bile duct; in such cases, if gallstones cause gallbladder disease (i.e., inflammation), pathological changes are likely to be present in both the gallbladder and cystic duct. Also, increasing lithogenicity of the bile, and other alterations in the inner environment [e.g., increased mucus production (86,98)], may influence the physiological state of the mucosa of the gallbladder or cystic duct or both, by decreasing its physical properties (e.g., resistance) and thus damage it. Lee and Scott (99) demonstrated pathological changes in the gallbladder before cholesterol gallstones had formed. A recent study of cholelithiasis has shown that pathological changes may not be uniform throughout the gallbladder and cystic duct, and thus has underlined the importance of examining both organs histologically (100). In the above study, the mucosa of the gallbladder was intact but the mucosa of the cystic duct contained multiple erosions (100), and the authors postulated that this cystic duct abnormality caused or contributed to subsequent gallbladder stasis.

The present study was designed to accomplish two aims: to evaluate the incidence and nature of cystic-duct disease associated with cholecystitis, and to correlate histological changes in the gallbladder body, neck, and cystic duct in cholecystitis. Because no uniform classification of gallstone disease has been established, a histological

scoring system was developed to define more clearly the grades of disease. This system is based on traditional classifications but allows quantification of the stage-related changes. In addition, it was designed to assess associations between individual changes in pathology and contractility.

Gallbladder Body and Neck

The normal histological structure of the gallbladder body and neck is generally similar, but there is less muscle and more elastic tissue in the neck (101): the neck's middle layer comprises mainly fibro-elastic components, whereas the body's is musculofibrous. Another feature distinguishing the neck from other parts of the gallbladder is its content of mucus glands, structurally similar to salivary glands; they are normally arranged singly, or in groups of two or three, in the areolar layer (101,102).

In this study, the histological abnormalities were qualitatively the same in both regions of the gallbladder but the hypertrophy associated with cholecystitis was more pronounced in the neck; the mucosal folds were taller, and in many cases the muscle layer in the neck was not much thinner than in the body, so that its relative increase in thickness was greater. The increase in the neck's muscular components lend some credence to the theory of a muscular sphincter in the inflamed gallbladder (28,101). This may have functional significance; it could also influence the rate of development of the pathological process in the gallbladder, as the excretion of bile requires synchrony and interaction of all parts of the bile-excretion system and disordered function in any part immediately affects the whole

system. Furthermore, any obstruction at the outlet will affect the degree of gallbladder inflammation. In cholecystitis, in most instances the obstruction is a calculus impacted in the neck or cystic duct, but in some it may be due to asynchronous activity of the 'muscular sphincter'.

Cystic Duct

Histological scores and pathological classification of the cystic duct were identical with those of the gallbladder, indicating that the cystic duct may be closely concerned in the etiology of gallbladder disease. Occlusion of the cystic duct has been considered a prime factor in the production of acute cholecystitis (103); this, of course, is commonly due to impaction of a gallstone in the duct's lumen. However, because the cystic-duct lumen is so small, and assuming that ductal and gallbladder histology are the same, minimal inflammation will have a much greater effect on the cystic duct than on the gallbladder. Minimal thickening of the gallbladder wall in 'early cholecystitis' is unlikely, on its own, to progress to more advanced disease; but equal thickening of the (narrow) cystic duct could delay gallbladder emptying and lead to stasis, and thus could induce a cycle of events leading to all degrees of chronic cholecystitis. It has been shown experimentally that bile retained in the gallbladder by cystic-duct obstruction induces inflammatory change and fibrosis of the gallbladder wall (104).

Mucus glands and epithelial appearances of increased mucus production were a common feature in the cystic duct in both human and canine specimens and present in the gallbladder body also in the latter. A recent study (98) indicated increased production of mucus in the pathogenesis of cholesterol gallstones. If hypersecretion of mucus

occurs in only the cystic-duct region in humans, it may be that inflammatory changes in the cystic duct predate those in the gallbladder.

As in other studies (84,85), fibrosis in the wall of the cystic duct was common, but this did not seem to limit the duct's contractility.

MUSCLE MORPHOLOGY

Hendrickson (26) and Lutkens (28) described a distinct circular band of muscle in the gallbladder neck. The present study revealed a definite muscle layer in the neck region, but on transverse section this appeared to be composed of longitudinal, circular, and oblique muscle fibers, similar in arrangement to the muscle layers in the gallbladder body and cystic duct. In a previous study (38) the mean thickness of the muscle layer in the human gallbladder revealed significant intergroup differences between gallbladders with mild chronic and advanced chronic cholecystitis, and between those with advanced chronic and acute cholecystitis. The muscle layer was thickest in advanced chronic cholecystitis (Table 3). Furthermore, comparison of muscle and wall thickness using a Zeiss MOP digital analyzer revealed a greater percentage of muscle in the body wall of gallbladders with advanced chronic than in those with mild chronic cholecystitis, and only a very thin layer of muscle was present in gallbladders with acute cholecystitis (38).

The muscle layer in the gallbladder neck was most hypertrophied in the human gallbladders with advanced chronic cholecystitis. In that

group the gaps between muscle bundles, which were filled with connective tissue, were narrow, whereas in mild chronic cholecystitis they were wide. In both human and canine specimens, the muscle layer was thickest in the gallbladder body, thinner in the gallbladder neck, (human) and thinnest in the cystic duct. These findings concur with those of Scott and Chansouria (29).

GALLBLADDER AND CYSTIC DUCT MOTILITY

Justification for Studies in Vitro

Studies in vivo are an essential element in acquiring knowledge of gastrointestinal motility, but they pose the problem of differentiating the effects of each of the many variables involved at any one time and are limited by ethics and technical difficulties. The investigation of motility in the diseased human gallbladder and cystic duct in vivo is further limited by regulatory effects of extrinsic nerves and hormones, changes in blood flow, and the influence of exogenous agents such as anesthetics (105) and other drugs. In addition, most studies involve radiological techniques that require patency of the cystic duct.

Experiments on isolated strips of tissue will eliminate many of these problems, and therefore provide a useful method for studying gallbladder and cystic duct contractility. Studies in vivo can measure gallbladder filling and emptying but they cannot evaluate the many components involved in these processes. This in vitro study was designed to investigate the relationship between cystic-duct motility and gallbladder contractility, two major components of gallbladder emptying.

However, this method of study, also, has limitations:

1. The lack of blood supply gives rise to two artefacts:
   (a) In inflammed and thickened tissue, the middle layers are probably hypoxic. However, for reasons unknown, strips of such tissue that are exposed to oxygenated medium respond for many hours.
   (b) Drugs introduced into the organ bath diffuse abnormally into the tissue (i.e, not from the blood stream). This may not be important, as studies of several substances with isolated tissue and in vivo have shown similar results (106). Many of the substances tested in motility studies of the gastrointestinal tract are present in the wall of the gut and, probably, of the gallbladder and cystic duct. As they are normally released within the tissue, diffusion into isolated preparations may simulate the conditions in vivo quite closely -- but diffusion into a tissue does not.
2. The effects of substances that act centrally cannot be detected on isolated tissues.
3. The effects of a drug in vitro and in vivo may be different; for example, a substance that is effective in vitro (e.g., acetylcholine) may be inactivated very quickly in vivo, and vice versa.
4. Biological idiosyncrasy always remains. This factor may be responsible at least partly for wide ranges of values in identical studies, both in vivo and in vitro.

Despite these limitations, the design of the present study allowed classification of changes in contractility in vitro and comparison with findings in vivo. Furthermore, as many of the test drugs exert their

effects directly on the muscle, contractile responsiveness could be correlated with the thickness of the muscle layer in various grades of inflammation.

Interpretation of Motility Results

There are many factors which can affect the responses of isolated gut muscle. There may be great variation in the way gut muscle from different species responds to drugs and different regions of the gut from the same animal may respond quite differently to a drug. The degree of oxygenation is important for the viability of the tissue, and often for the level of tone and spontaneous activity. The temperature, recording techniques, and the storage of tissue can all affect the responses. Sensitivity of isolated tissues to drugs often fluctuates during the course of an experiment and declines markedly after several hours. Submaximal control responses to constant doses of drug must therefore be obtained during the course of an assay. The responses of isolated smooth muscle are influenced by the age and sex of the animal. In this study on pathological tissue, the thickness of the strips may affect the sensitivity to the agonists by slowing the rate of diffusion of the drugs to the receptor sites on the muscle.

When studying pathological tissue, one must also take into account that real changes in sensitivity to an agonist (as distinct from apparent changes) may occur due to receptor damage. This conceivably could happen in cholecystitis where the muscle is often involved in the inflammatory process. It is therefore important to look directly at the binding of drugs to their receptors rather than inferring the binding affinity from an analysis of dose-response curves. One of the simplest

and yet most direct uses of receptor binding studies is the determination of the number of receptors in tissues, and the changes in receptor density during development, and as a result of neurochemical and pathological lesions.

The above-mentioned factors serve to illustrate the problems associated with the measurement of biological responses to drugs. Despite these problems bioassay is often more sensitive and more specific than existing methods of chemical and physical estimation, and it is simple and inexpensive. In this study adherence to a rigid protocol allowed for more accurate interpretation and comparison of results.

TONE AND SPONTANEOUS ACTIVITY

Intestinal smooth muscle possesses intrinsic myogenic tone which is not dependent on nervous stimulation; i.e., it occurs spontaneously even in isolated nerve-free preparations It is a feature of most types of smooth muscle. Myogenic excitation originates spontaneously in a group of muscle cells acting as pacemakers. This type of myogenic tone has been observed in all parts of the biliary tract (42,107).

When isolated smooth-muscle preparations are set up in organ baths, there is intrinsic tension in the tissue. In addition, the tissue can exhibit rhythmical contractile activity; this often increases with time and following the addition of drugs to the organ bath. These features have been observed in isolated canine and guinea-pig gallbladder strips (42,107). In this study both human and canine gallbladder and cystic duct strips exhibited spontaneous rhythmical activity in vitro. However the precise characteristics of this activity varied in both

organs. This observation suggests that there is a gradual change in the character of the spontaneous activity from gallbladder to cystic duct. The significance of this finding is unknown.

Little is known of the electrophysiological events underlying tone and spontaneous activity in the biliary tract, which may not be comparable to those in other parts of the gastrointestinal tract.

Acetylcholine

The results of the present experiments make it clear that the isolated human and canine gallbladder and cystic duct muscle possesses cholinergic receptors. We found that the vast majority of the strips responded to acetylcholine. The responses are probably not affected by premedication or anaesthetic drugs. As Fishlock and Parks (108) found that variations in premedication and anaesthesia made no difference to the responses of colonic muscle to acetylcholine, it seems likely that results obtained with tissues removed at operation represent the pharmacology of untreated isolated tissue.

Acetylcholine, which acts directly on muscle cells, always caused contractile responses. The responses were similar in most respects to those of intestinal smooth muscle, though the time course of the contraction was slower in most cases.

Clanachan *et al.* (40) have demonstrated increased resistance to flow in canine cystic duct following acetylcholine stimulation and have also shown acetylcholine-induced contractile responses in the cystic duct *in vitro*. Their findings *in vitro* concur with ours, and lend support to the contention that the cystic duct has sphincter-like properties.

Cholecystokinin

The recent development of a more specific and reliable radioimmunoassay for CCK (109) and of gallbladder imaging by ultrasonography and cholescintigraphy has provided the tools for safe, detailed study of CCK's physiological effects. These noninvasive techniques will enable studies of gallbladder emptying in healthy human volunteers and patients with gallstones, and the *in vitro* technique reported here will permit evaluation of the effect of CCK on contractility of gallbladder and cystic duct, a major component of the emptying process.

It is assumed that CCK relaxes the sphincter of Oddi. This assumption, together with the belief that CCK's only mode of action in the biliary tract is via a direct effect on smooth muscle (60,61,110) has led to the presumption that CCK, if it affects the cystic duct, relaxes it. All of the relevant studies have shown that CCK contracts smooth muscle (59,60,110). However, Toouli *et al.* (54) recently postulated that the octapeptide of CCK (CCK-OP) may also stimulate nonadrenergic, noncholinergic inhibitory nerves within the sphincter of Oddi, and that this may be its dominant effect. If, therefore, the sphincter relaxes when the direct stimulatory effect of CCK on muscle is overridden by its neurally mediated relaxant effect, it is not unreasonable to suppose that absence of inhibitory nerves in the cystic duct, or a minimal effect of CCK on them, would render CCK's dominant effect on the cystic duct contractile.

The contractile responses of the human and canine cystic duct to CCK-OP in this study concur with the findings, both *in vivo*, and *in*

vitro, reported by Courtney *et al*. (62). Also, their study revealed a differential sensitivity to CCK in the gallbladder and cystic duct. The sensitivity to CCK was up to six times less in the duct than in the gallbladder, and responses to CCK *in vitro* were more sluggish in the duct. In the present study of human specimens, the sensitivity difference to CCK of the duct was only three times less than in the gallbladder and the mean maximal response to CCK, through smaller in the cystic duct than in the gallbladder, was not significantly so. Thus the dynamics of bile flow in humans are more likely to be determined by sensitivity differences to the agonists that control extrahepatic biliary motility than by the magnitude of the responses. These findings in human tissue tend to support the hypothesis of increased resistance to bile flow in the cystic duct in patients with gallstone disease. Interestingly, two of the human gallbladders contained pigment stones (one patient had hereditary spherocytosis), and in both cases the sensitivity difference between the gallbladders and cystic ducts was only two-fold. (In one normal human gallbladder not included in the present study, the cystic duct was 12 times less sensitive than the gallbladder to CCK).

The stimulatory effect of CCK on the cystic duct may help to explain the motility abnormalities in symptomatic acalculous gallbladder disease. Cholecystokinin cholecystography is used in some centers to detect motility disorders of the gallbladder (112-114) because it provides a maximal stimulus to contraction of gallbladders shown by conventional cholecystography to empty very slowly. In two studies with CCK cholecystography (91,113), visualization was prolonged and emptying of the gallbladder was delayed. I have formed the hypothesis that, in

this group of patients with functional biliary-tract disease (e.g., cystic-duct syndrome, biliary dyskinesia), the difference in sensitivity to CCK between the gallbladder and cystic duct is minimal - or even reversed, so that the cystic becomes more sensitive to CCK than the gallbladder. As studies of these organs from two such patients (not in the present study) showed sensitivity to CCK to be 3 and 5 times greater in the cystic ducts than in the respective gallbladders, this hypothesis appears at least as plausible as those proposed by others (113-115), including kinking of, compression of, or abnormal valves in the cystic duct, or degenerative diseases of the gallbladder. In a majority of cases, there is also some degree of gallbladder inflammation (83,115), most likely the result of gallbladder stasis due to the impaired gallbladder emptying just described.

Evaluation of the CCK-OP-induced contractile responses in the gallbladder neck showed mean sensitivity and mean maximal response to CCK-OP the same as in strips of the gallbladder body. This partly refutes the postulated existence of a physiological sphincter in the gallbladder neck. There was no gradual transition in sensitivity to CCK-OP between the body of the gallbladder and the cystic duct.

MEDIATORS OF INFLAMMATION

Histamine and bradykinin can be termed local mediators of inflammation. The formation or release of such mediators in response to a stimulus, such as cell injury or antigen antibody reaction, is not well understood. As local mediators are usually inactivated before producing systemic effects, their distribution is probably an important

determinant of their role. For example, as most of the body's histamine is in mast cells (116), the distribution of histamine relates to the location of this type of cell; (this is usually in close proximity to small blood vessels) as mast cells are present in almost all tissues, histamine can exert its effect anywhere in the body. Similarly, as the system that forms bradykinin is present in all tissue fluids throughout the body, bradykinin can be activated at any site (117).

Therefore, both these local mediators may be involved cholecystitis and, as a result, may affect gallbladder and cystic-duct motility.

Histamine

Both H_1 and H_2 histamine receptors are present in the cystic duct (5). In the canine cystic duct, the predominant effect of histamine is an H_1 -receptor-mediated increase in resistance (5). Many investigators believe histamine helps modulate the motility of the extrahepatic biliary tract (64-68).

In this study, histamine contracted both gallbladder and cystic duct in both species. Although the degree of sensitivity to histamine was the same in both organs, the rate of contraction and the time required for maximal contraction appeared to differ, responses being slower, and their magnitude significantly lower, in the cystic duct. There was no change in the sensitivity to histamine of the human gallbladder neck strips, but the magnitude of the responses in the neck were intermediate to those of the gallbladder body and cystic duct. Therefore it is possible that the gallbladder contracts strongly, initially, and that delayed, weaker, contractions occur in the neck and cystic duct. Thus, any effect of histamine on contractility of the

extrahepatic biliary tract does not prevent expulsion of bile from the gallbladder.

Bradykinin

The range of sensitivity of both human and canine tissues to bradykinin was very narrow. In the canine specimens, sensitivity of the cystic duct and gallbladder was the same. In the human tissues, sensitivity of the gallbladder neck and cystic duct to bradykinin was the same, and significantly greater than sensitivity of the gallbladder body. However, the difference was only two-fold; this is very small in an *in vitro* study, casting doubt on its significance in modulating biliary-tract activity.

Although it is difficult to ascribe a role to bradykinin in motility of the gallbladder and cystic duct, this substance is a potent stimulant of both organs. As a local mediator of inflammation, if present in large-enough amount it might give rise to both quantitative and qualitative differences in activity. The concentration of a mediator represents the balance between amount formed and amount inactivated. In cholecystitis, damage and anoxia result in acidity of the tissues. At an acid pH, the enzymes that inactivate bradykinin are inhibited whereas those that form it are little affected; thus an acidic environment favors the accumulation of bradykinin, which therefore is likely to be present in both gallbladder and cystic-duct tissue in disease. If the gallbladder neck and cystic-duct region were more acidic in cholecystitis than the gallbladder body, this might explain

the sensitivity difference to bradykinin noted above. However, bradykinin may have no direct role itself, only modifying the effects of other agonists on the tissues (78-81).

CONCLUSION

In 1937, Lichtenstein and Ivy (10) concluded from their observations: "The cystic duct is devoid of any organized system of musculature, which by itself could by contraction produce changes in the internal pressure in the cystic duct". This assertion by the leading investigators of the biliary tract led to neglect of cystic duct function for many years. Following on the recent renewed interest in this organ (3-7,11,29,62), the present study has given clear indication that the cystic duct is an active component of bile-flow-dynamics.

The observations reported here showed a distinct correlation between cytic-duct and gallbladder pathology in gallstone disease and indicated an etiological link between cystic-duct disease and stasis of bile in the gallbladder. Further investigations are necessary to determine whether stasis as a result of anatomical, pathological, or functional abnormalities of the cystic duct, or a combination of these, is responsible for gallstone formation in humans.

The contractility studies demonstrate that the cystic duct is influenced by the same neuro-hormonal mechanisms as the gallbladder. In gallstone disease,mediators of the inflammatory reaction may assume an important role in both gallbladeer and cystic-duct motility. Motility of the cystic duct requires more attention; the duct appears able to respond in unison with or contrary to the gallbladder. Therefore it can function independently despite its structural attachment.

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